CALCIUM SIGNALING: KYIV HISTORY

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Platon Kostyuk, *M.D., Ph.D., D.Sc., born on August 20, 1924. He received his M.D. in 1949 from Kyiv Medical Institute and started his experimental work under the supervision of Prof. D.S. Vorontsov. In 1958 he developed by himself the microelectrode technique for intracellular recording spinal motoneurons and presented his findings at the International Physiological Congress in Buenos Aires. After that he was invited by J. Eccles to the Australian National University in Canberra, where in 1961 he together with R. Schmidt performed extensive studies on the mechanism of presynaptic inhibition and with M. Ito on the ionic mechanism of postsynaptic inhibition. This line of investigation continued in Kyiv at the Bogomoletz Institute of Physiology of the Ukrainian Academy of Sciences, where he founded and headed the Department of General Physiology of Nervous System. Here he started a new line of researches devoted to ionic mechanisms of nerve cell excitability. An important step in this direction was the construction together with Z. Sorokina intracellular pH-electrodes. In 1975 a technique for intracellular perfusion was developed jointly with O. Kryshtal and V. Pidoplichko, which opened excellent possibilities for separation of different components of transmembrane ionic currents in neuronal membranes. Detailed characteristics of calcium currents as well as analysis of the gating and selectivity mechanisms of the corresponding channels were reported for the first time. In 1984 the presence of different types of voltage-operated calcium channels was detected in membranes of different neurones. P. Kostyuk and his collaborators carried out extensive investigations on the activity of single ion channels in different excitable cells, changes in channel functioning during cell development and differentiation, mechanism of channel modulation by different intracellular messenger systems, expression of ion channels in oocytes after mRNA injection, etc. These data have been summarized in several monographs, among them "Intracellular Perfusion of Excitable Cells" 1984; "Calcium ions in nerve cell function" 1992; "Calcium signaling in the nervous system" Kostyuk & Verkhratsky 1995; "Plasticity in nerve cell function" 1998. He was a member of Russian and Ukrainian Academies of Sciences, European Academia, and a holder of several international scientific awards.*

The idea of a particular role of calcium ions in the maintenance of basic functional properties of living cells was expressed almost a century ago. As it has been noticed by the pioneering observations of Ringer (1873) and Loeb (1906), a definite relation between monovalent (sodium) and divalent (calcium) in the external medium is necessary for the execution of active cellular reactions. The way to the understanding of the mechanisms of this crucial role of Ca ions has been long and complicated, and our group has quite effectively participated in it, and here I would like to remind briefly how it happened.

As we remember, in the 50s of last century our connections with foreign laboratories were almost blocked. This was the time of fast progression of intracellular microelectrode techniques, and we developed them by ourselves (Kostyuk, 1960). Quite lucky such recordings from spinal inter neurons were presented in 1959 at the International Physiological Congress in Buenos-Aires and listened by Eccles. First, he did not believe that such recordings were made by ourselves, but later invited me to work in his department in Canberra (Australia) in collaboration with prominent neurophysiologists (Schmidt, Willis, Ito). This collaboration extremely amplified my possibilities in the analysis of ionic processes in different types of nerve cells and resulted in several papers in prominent journals.

This origin and development of investigations of ionic processes in single cells started in Kyiv in a small laboratory belonging to Kyiv State University, not to the main research institute of the NAS Bogomoletz Institute of Physiology, whose 75th anniversary of foundation is celebrated this year. Bogomoletz transformed an empty hill in the center of our city into a beautiful park where the institute became located. Its main research directions were physiology and pathology of the cardiovascular system, immunology, and other systemic functions. Only in 1956 the department of electrophysiology was organized in this institute by Vorontsov, my teacher at the university. Later this opened for me the way to this excellent academic institute and to organize in it the Laboratory of General Physiology (1958), which with time rose to a department.

The main topic of our investigations that time was a comparative analysis of the function of Ca^{2+}-selective voltage-operated ion channels in different excitable cells which form the main pathway for transmembrane calcium currents. An important technical step was the creation of a special technique allowing recording of calcium currents separately from other types of currents (sodium, potassium), namely the intracellular perfusion or dialysis (Kostyuk et al., 1975; Kostyuk et al., 1977). Thanks to using this technique, it became possible to precisely characterize calcium channels in different types of cells. In our group the presence of two main subtypes of such channels was detected; they were divided into low- and high-voltage activated ones with quite different functional characteristics (Veselovsky and Fedulova, 1983). This division has been confirmed in other laboratories (Carbone and Lux, 1984; Nowycky et al., 1985). High-voltage activated channels were then divided into steady-state and

activating ones, and symbols proposed by Nowycky for all these channels (N, L, T) are still widely used.

The introduction of the intracellular perfusion technique opened for us the way to intensive investigations of biophysical properties of calcium channels and intracellular mechanisms determining the function of Ca^{2+} ions entering the cytoplasm ("calcium signals"). We established the unitary conductance of different types of channels (Kostyuk et al., 1988) and possible mechanisms determining their conductance specificity. The presence in them of two separate binding sites was predicted in (Kostyuk et al., 1982). A striking feature of the channels, namely immediate loss of selectivity after removal of divalent ions from the external medium, was detected, which may indicate that another high-affinity binding site is present in the channel molecule that normally is occupied by divalent ions. When they are taken away, a conformational change occurs modifying the energy profile of the channel and transforming it into a sodium-permeable one (Kostyuk and Mironov, 1986).

Calcium signals produced by activation of ligand-activated channels are another important research topic of our group. In this respect, our interest is concentrated specially on purinoreceptors activated by extracellular ATP (discovered by Kryshtal, Marchenko, and Pidoplichko (1983). An extensive study of these receptors on different neurons demonstrated the important role of calcium influx through channels in modulation of synaptic transmission, complex interaction of such influx with activity of voltage-operated calcium channels and possible effects of opioids. To continue this topic, a search for the sources of extracellular ATP was required.

The fate of calcium ions which enter the cell is another important question extensively studied in many laboratories. Our interests were focused on the determination of expression of these mechanisms in different parts of the cell and different cell types. The endoplasmic reticulum and its two molecular structures which trigger Ca^{2+} release were first studied (Verkhratsky et al., 1994). An important finding was the age dependence of this mechanism. Reduction of the calcium signal amplitude was found in aged neurons, accompanied by prominent deceleration of the cytoplasmic Ca^{2+} level recovery — an alteration of its extrusion mechanisms. Retardation of calcium accumulation by endoplasmic reticulum was demonstrated as one of the reasons for such changes. Such alterations may be responsible for the increased vulnerability of aged brain.

It is very interesting that aging in our investigations was found to be very specifical in the functioning plasmalemmal calcium channels. The conduction of high-voltage activated channels remained identical in neurons of newborn and aged animals. But the low-voltage activated calcium channels disappeared completely during aging, while the density of high-voltage activated ones only decreased. This obviously indicate that calcium influx through low-voltage activated channels play a special role in the period of development of the nervous system.

The role of mitochondria in determination of the fate of intracellular Ca^{2+} was also extensively analyzed by our team. The mechanisms of uptake and release of Ca^{2+} were determined. In fact, mitochondria were the first intracellular organelles associated with the regulation of calcium homeostasis, which was summarized in (Carafoli and Crompton, 1976). Our team got also interested in possible role of mitochondrial calcium manipulations during frequent pathological processes. One of them is diabetic naturopathy connected with increasing pain syndromes. An important finding obtained on rats with experimentally induced diabetes was substantial prolongation of calcium transients in primary nociceptive neurons leading to potention of synaptic transmission of secondary neurons. In parallel, a diminution of calcium uptake and substantial prolongation of its release as back to cytosol by mitochondria and endoplasmic reticulum were recorded leading to the described changes in calcium signaling (Kostyuk et al., 1999; Kostyuk et al., 2001). We also studied such brain pathologies as epilepsy. The role of N-type calcium channels in the mechanisms of action of the antiepileptic drug has been shown (Lukyanetz et al., 2002). Our group demonstrated the participation of calcium channels in the pathological effect of hypoxia on nerve cells (Lukyanetz et al., 2003). The involvement of calcium channels in the development of Alzheimer's disease has been shown (Korol et al., 2009).

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